

any very great difficulty at New Norfolk river : it would require only to take or kill a female *Ornithorhynchus* at the latter part of September, and repeat the capture of other females during each week of the months of October and November, or in December if the specimen taken at the end of November was still pregnant. The hinder half of each of such specimens, with the female organs, or simply the impregnated uterus, should be preserved in strong colourless spirits ; and if this should meet the eye of my esteemed correspondent Mr. Ronald Gunn, or of Dr. Casy, I would earnestly solicit their kind co-operation in transmitting such specimens to me at the Royal College of Surgeons, London.

XXXV.—*An Account of the Germination of Isoëtes lacustris.*

By KARL MÜLLER*.

[With two Plates.]

[Continued from p. 188.]

6. *Formation of the Third Leaflet.*

IN order to make the appearance of the third leaflet particularly clear, I have given an illustration of the development of a single individual up to this point, as it may be seen either by the unassisted eye or under a lens ; the embryos of fig. 24 *a-g* represent this. I do not think it necessary to enter again upon the details of the earlier stages, and I pass now at once to the actual appearance of the third leaflet.

After I had made many conjectures as to the point where this leaf would appear, it was formed, in the most fitting place, in the middle, between the first and second leaves. Like these two it issued gradually as a very delicate cone, the cells of which speedily became more firm the more it came to light. The formation, as a whole, had nothing else particularly striking about it.

I was now extremely anxious to know how the third root would be developed. With all my many embryos I have not attained the satisfaction of becoming acquainted with this point, since all died in this stage. I here communicate my experience of the rearing of *Isoëtes* to botanical gardeners, in order to spare them the vexation of seeing their young, hopeful crop all die away in this stage. Success will be most surely attained by using a glass of considerable size instead of a pot ; which must be filled pretty

* Translated by Arthur Henfrey, F.L.S., from the *Botanische Zeitung*, May 5, 1848.

high up with clean sand, the ripe ovules strewed upon this and loosely covered by a thin layer of sand. Germination is sure to follow. If the lower portion of sand be deep enough, the young roots penetrate down with great strength into this layer; and if the glass be high enough to allow of its being filled up with a considerable depth of water, the young leaflets also grow upward with great rapidity. These deep strata of water and of sand act like a wonderful charm upon the young embryos. If these however cannot attach themselves very firmly by their roots, they come to the surface of the water, and then it is impossible to set them again so as to make them thrive any longer. They are sure to die.

The reason why I wished so much to observe the formation of the third rootlet was the desire to solve, by indubitable proofs obtained from the course of development, the question once propounded by H. v. Mohl, as to the reason why the youngest roots of the rhizome of *Isoëtes* occur in the centre and the older in the periphery.

I cannot settle this question from the experience of my embryos, yet I believe that it may be brought near to a decision through fig. 24 *d*.

There is no question that the root of the third leaflet must be developed in a different way from the two preceding. These must be formed outwardly, since the leaves which belong to them lie toward the exterior. But the third leaf lies in the interior, between the two first leaflets (fig. 24 *d*, *x*); consequently its root must also be formed in the interior. Now since the vascular bundle of the leaf constantly ran into the root in the two first leaflets, it is probable that the vascular bundle of the third leaf will do the same. Then it must penetrate the rudiment of the young rhizome and make its appearance as the youngest root, between the two older, consequently in the centre of the rhizome. Under these circumstances, we have to wonder less at the peculiar mode of issue of the root than at the phenomenon that the vascular bundle belonging to every leaf is elongated out from it through the rhizome downward, and there comes to light as a root. The latter consequently has a wholly dependent connexion with the leaf. We can therefore as little talk of a principal root in *Isoëtes* as in the *Selaginellæ*, in spite of Schleiden's opinion.

A. Braun some time ago* also gave an explanation of the peculiar arrangement of the roots in *Isoëtes*. He said that it might readily be explained if it were assumed that the vessels, in-

* Flora, 1847, No. 3.

stead of breaking forth outward from the vascular cylinder, turned inward and perforated the rhizome. According to this therefore a proper vascular cylinder must be present in the rhizome, and as such must be understood the internal medullary layer*. This is not clear to me. From Mohl's illustrations I do not know in what relation the vessels, occurring in the lower part of the medullary layer, stand to the leaves. According to these figures the upper vessels most certainly stand in connexion with the leaves, and they admit of its being conceived that the above ground of explanation which I have deduced from the course of development actually hits the fact; that the vessels of the youngest roots correspond to the youngest leaves.

The gradual death of the young embryos appeared to me very peculiar: I have represented it in the figures (fig. 24 *e, f, g*). The root of the first leaflet went first, and the coats of the primine with the secundine still adhered to the first leaflet (fig. 24 *e*). Then this also was lost (fig. 24 *f*), and by this means the third leaflet became more free. Next the root of the second leaflet disappeared (fig. 24 *g*), and after that the leaf itself. At last only the third leaflet remained: this lived, fresh and growing for some time; but the true vital foundation appeared to have departed from it, and after several weeks' vegetation it died like the rest.

Now that we have become fully acquainted with the course of development of *Isoëtes* up to this point, I consider the observation of the further progress will be very easy, if any one, in whose neighbourhood *Isoëtes* occurs, will take the pains to fish up young embryos out of the water, and continue the examination from the point at which I have ceased. The embryos are probably not difficult to find in their natural condition. I imagine that they must grow socially, in great numbers close together, since they will in any case germinate simultaneously out from the oophoridia of the decaying parent plant, as often happens in aquatic plants.

7. Retrospect.

A. Special course of development of *Isoëtes*.

1. The foundation of all cell-formation of the embryo is a delicate granular mass which exists very early in the ovule and completely fills it (fig. 4).

2. This mass becomes transformed into starch, and is thus brought into a condition for solution (fig. 5).

* See the illustrations, by H. v. Mohl, *Linnæa*, 1840, t. 3. (Also in *Vermischt. Schrift.* plate 5.)

3. The dissolved starch takes the appearance of oil-globules, and these alone constitute the *protoplasm* of the first cells of the embryo (fig. 6).

4. The embryo, which is already formed at a period when no alteration is to be observed in the coats of the ovule, first appears as a solitary cell.

5. It becomes a mother-cell, new daughter-cells being soon formed within it. This certainly is effected through cytoblasts (figs. 7, 8).

6. Soon after this the mother-cell has become a wholly cellular, oval corpuscle (figs. 9, 10).

7. Hereupon, a growth toward two sides is seen at the base, and the corpuscle becomes curved in the middle, in which a solitary cell displays itself: this is the rudiment of the scale of the first leaf. The growth toward two sides marks the rudiments of the vagina and alimentary organ (fig. 11).

8. Two more organs are now associated with the preceding. The alimentary organ becomes more evident by growing out in a rounded form; through this originates a curve on the base of the embryo which is also partly caused by the root of the first leaf, which is equally visible in its rudimentary condition. By this also the vagina becomes directed upward, it is connected with the future furrow of the leaf, and incloses, together with the mother-cell of the first scale, the newly added one of the second leaflet (fig. 12).

9. The first leaf having become cylindrically elongated, it has also acquired a green colour. At this time the alimentary organ, root, vagina, scale and mother-cell of the second leaflet are clearly distinct organs. The alimentary organ, which occupies one-half of the germinal body while the other half belongs to the radicle, has become filled with starch which suffices for the nutrition until the second leaflet has become tolerably developed. The root is still a little conical body formed of very delicate cellular tissue. The vagina looks sideways, and incloses now the two mother-cells of the scale and the second leaflet. The former becomes a flat membrane, the latter remains a round, but internally already cellular body (figs. 15, 17).

10. The scale and root alone now visibly hasten forward in their formation. The former has attained its full development, while the second leaflet has only become cellular and the vagina applied itself more closely upon it. The root has most visibly advanced; it has become considerably elongated and acquired two vascular cords (fig. 18).

11. The next succeeding development of all parts is merely a relative expansion of them (fig. 20).

12. The vagina has merely become a very delicate membrane, and the vascular cords of the root united into one mass at that time when the second leaflet issues from the vagina as a green, somewhat flattened corpuscule. The root now expands only at its apex by cell-formation, since its only point of vegetation lies there (fig. 21).

13. When the second leaflet has issued from the vagina, which now forms a long, delicate membrane obliquely cut off at its open end, the starch of the alimentary organ has disappeared. The vascular bundle of the first root has again separated into two portions; each portion however consists of several, usually two cords. The root of the second leaflet now shows itself, at first only as a very transparent compact protuberance (fig. 22).

14. The further development of this radicle appears to have become the principal matter in the whole plant. As in the first root, the vessels are first formed as two cords (fig. 23).

15. Then they become elongated, like those of the first, with all the gradations of the preceding formation (fig. 24 b).

16. After the second root has attained its full development, the third leaf makes its appearance between the two first (fig. 24 c, d).

B. Comparison of the course of development of *Selaginella* and *Isoëtes*.

No comparison is possible between the earliest stages of development of these two genera, since those of *Selaginella* are yet unknown. Nevertheless they perfectly agree in one point,—that the element of cell-formation is in both a granular mass, which becomes converted first into starch and then into protoplasm. At this point our comparison is terminated, and we begin again at a considerably advanced period of development, in the young plant.

The young embryo of *Selaginella* consists, like that of *Isoëtes*, of three essential parts: 1. the alimentary organ; 2. the radical portion; 3. the terminal bud.

The alimentary organ is here a special body originally very imperfectly, but in further-developed states of the plant perfectly round. This alone remains within the ovule, to attach the little plant to it (fig. 25 a). No starch is deposited in its interior; the protoplasm which must be formed from the granular mass (fig. 25 d) still existing within the ovule, is taken up by it and carried onward to the remaining portions of the embryo. In this variation this body does not wholly represent the organ in *Isoëtes*. The most peculiar point in it however is, that it does not become wholly rounded off until a subsequent period.

The radical portion does not lie, as in *Isoëtes*, with the alimen-

tary body, inside the ovule, but outside this, beside the terminal bud (fig. 25 *b*) : therefore the ovule is never perforated by a root as in *Isoëtes*.

The most important distinction between the genera, is that in *Selaginella* an actual terminal bud is produced, in *Isoëtes* none, which is naturally explained by the fact, that in *Selaginella*, elongated axes are developed, while in *Isoëtes* the axis always remains short and appears as a rhizome. On this account also the individual organs are never produced symmetrically here in *Isoëtes*, as they are so strictly in the terminal bud of *Selaginella*.

These may be taken as the most essential points which allow of comparison in the young embryos of the two genera. We trace in them all the similarity which may be concluded from the relationship, but also all the dissimilarity that has its ground in the different type of the two genera, which is at once expressed in the course of development. The most essential relationship between the two, however, is above all the formation of the embryo in the interior of an ovule, without a pollen-tube.

It is exceedingly desirable that, in order to complete our knowledge of the mutual relationship, some one may be able very shortly to furnish the history also of the earliest stages of development of the *Selaginellæ*.

Possible Results.

After it has been shown in the foregoing how a cell is able to form in an independent manner, inside a perfectly closed organ, without a pollen-tube ! I might repress all further conclusions against those who derive the formation of the embryo of sexual plants from a constricted pollen-tube. But I have one more observation on *Isoëtes* to subjoin, which I could not pass over in silence, since it appeared to me to possess great importance in regard to that controversy.

It is well known that in many plants two embryos sometimes appear within one and the same ovule. This phenomenon is explained by Schleiden by the penetration of two pollen-tubes. I have observed the same phenomenon in *Isoëtes*, where two independent embryos had been formed in one and the same ovule, and I have represented the case in fig. 19. It was evident however that neither of the embryos were in a good condition ; though they were normally formed, both suffered from it, in various places. One was developed considerably at the cost of the other, and possessed two vascular cords in its root. Nothing of these could be seen in the other. It would have fared badly with both also, afterwards, since neither of them had any of the starch, the so necessary nutriment, in the alimentary organ (fig. 19 *e*).

But it is perfectly clear from all this that so many pollen-tubes are not necessary for the formation of any given number of embryos.

The formation of the embryo in *Isoëtes*, or in the *Lycopodiaceæ* generally, must also be of interest in other ways in regard to that of the sexual plants. In *Isoëtes* no pollen-tubes penetrate, and yet the embryo-cell is developed independently. In the sexual plants a pollen-tube must first have entered, before that cell can be formed. I here of course assume that the indulgent reader agrees with me that the germinal cell first originates in the embryo-sac after the penetration of the pollen-tube in the sexual plants; in favour of which I have already elsewhere furnished my observations*. This appears to me capable of explanation in a consequential manner. In *Isoëtes* the ovule contains the true *protoplast*, from which may be developed a peculiar mother-cell like that of the embryo; in the sexual plants this matter is not present in the embryo-sac. It must be furnished in a different way and is brought by the pollen-tube.

In conclusion, let me hope that I have succeeded in the foregoing pages in exhibiting, fulfilled and completed, the three principal objects of these investigations, which I mentioned in the introduction: how far *Isoëtes* and *Selaginella* are allied in their course of development; how the peculiar formation of the root takes place in *Isoëtes*; and how the uterine formation of the embryo in an asexual plant is related to that of the sexual plants.

EXPLANATION OF PLATES II. & III.

N.B. The Numbers 5, 20, 50, 250 and 400 give the enlargement of the object.

Fig. 1. An ovule with the two outer coats just burst, and the coat of the nucleus (*a*) visible.

— 2. This last magnified to show its peculiar structure.

— 3. The great papilliform cell with the contiguous cells, magnified.

— 4. The granular contents of the ovule.

— 5. The same converted into starch.

— 6. The latter transformed into protoplasm.

— 7. *a, b.* The first or embryo-cell.

— 8. *b.* The same. *c.* A free cytotblast. *d.* A cytotblast around which the membrane is formed.

— 9. *a, b, c.* The embryo-cell now become a cellular corpuscule. *c.* Treated with iodine to show how the cell-membrane of the mother-cell incloses the cellular contents like a sac.

— 10. *a.* The embryo, becoming elongated. *b.* A single cell with cytotblastema.

— 11. The same. *a.* Alimentary organ. *b.* Vagina. *c.* Cell of the first scale; all just begun to be developed.

* Bot. Zeitung, 1847. Beit. z. Entwicklungsgesch. d. Pflanzen-embryo, p. 737 *et seq.*

- Fig. 12. Further stage. *a*. Alimentary organ. *b*. Radical portion. *c*. Mother-cell of the second leaflet. *d*. Vagina. *e*. First leaf. *f*. Cell of the first scale.
- 13. The same, drawn from the face. *a*. A cell which is the support of the cell of the first scale. *b*. Mother-cell of the second leaflet. *c*. Cells of the vagina. *d*. Cell of the first scale.
- 14. Ovule bursting open. *a*. Primine. *b*. Secundine. *c*. Nucleus.
- 15. *a*. Ovule, when the young embryo has just broken through the apex of the coat of the nucleus; the young embryo shines through, of a green colour, at the summit of the coat of the nucleus. *b*. The young embryo extracted. *c*. Alimentary organ: *d*. Cell of the first scale. *e*. Mother-cell of the second leaflet. *f*. Radical portion. *g*. Vagina.
- 16. *a*. Ovule, when the embryo has entirely broken through the apex of the coat of the nucleus and issued all except the germinal body (pro-embryo). *b*. The little plant extracted: this is otherwise not normally formed: the alimentary organ and radical portion are not yet visible. *c*. First scale. *d*. Cell of the second leaflet.
- 17. *a*. Embryo elongating by the expansion of its cells, extruded from the coat of the nucleus as far as the germinal body. *b*. The same magnified 250 times. *c*. First scale. *d*. Second leaflet. *e*. Root. *f*. Alimentary organ. *g*. Outermost portion of the root, in the cells of which all the contents have become fluid and transparent.
- 18. *a*. Embryo of the natural size. *b*. 250 times enlarged. *d*. Alimentary organ. *e*. Apex of the root. *f*. Vessels. *g*. Epidermoid outermost layer of cells of the root, with cytotlasts. *h*. First scale. *i*. Cells of the vagina. *k*. Second leaf.
- 19. *a*. Two embryos in a single ovule, of natural size. *b*. Magnified 20 times. *c*. Magnified 50 times. *dd*. Second leaflets, *ee*. Empty alimentary organs.
- 20. *a*. Embryo, nat. size, when the root breaks through the lowermost part of the coat of the nucleus. *b*. Magnified 50 times with *g*, the extruded root. *c*. Extracted. *d*. Second leaf. *e*. First scale. *f*. Alimentary organ. *h*. Vagina.
- 21. *a*. Embryo prepared free from the ovule; 50 times enlarged. *b*. Spiral vessel, 400 times enlarged. *c*. Alimentary organ. *d*. Second leaflet. *e*. Point of vegetation on the apex of the root. *f*. End of the spiral vessel in the root. *g*. Vagina.
- 22. *c*. Empty alimentary organ. *d*. Vagina. *e*. Second leaflet. *f*. Second radical portion.
- 23. *a*. Embryo, nat. size. *b*. Magnified 50 times. *c*. Empty alimentary organ. *d*. Vagina. *e*. Second root.
- 24. Development of an individual up to the formation of the third leaflet. *a*. First leaf. *b*. Second leaf. *c*. Third leaf. *d*. The same magnified 50 times with *x*, the third leaf. *e*. The stage when the plants died: first root gone. *f*. First root and first leaf gone. *g*. First root, first leaf and second root gone.
- 25. Germinating plant of *Selaginella*. *a*. Germinal body (pro-embryo). *b*. Radical portion. *c*. Terminal bud. *d*. Granular contents of the ovule.